INTERACTION OF SOMATOSTATIN AND CALCIUM IN REGULATING INSULIN RELEASE FROM ISOLATED PANCREATIC ISLETS OF RATS.

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SUMMARY

The effect of synthetic somatostatin on insulin release was studied in vitro by using isolated islets of rats. Somatostatin, with concentrations from 10 ng/ml to 10μg/ml, inhibited insulin release induced by 16.7 mM glucose. Insulin release elicited by 10μg/ml glucagon or 2 mM dibutyryl cyclic AMP was likewise inhibited by 100ng/ml somatostatin. By raising the calcium concentration of the incubation medium to 6 mM, glucose-induced insulin release was fully restored even in the presence of somatostatin. However, the same maneuver only partially counteracted the somatostatin inhibition of dibutyryl cyclic AMP-induced insulin release. These results suggest the involvement of calcium mobilization process in the inhibitory action of somatostatin.

INTRODUCTION

Somatostatin, a growth hormone release-inhibiting hormone, has been found to inhibit insulin release elicited by various secretagogues in vivo (1, 2). However, the inhibitory effect of somatostatin on insulin release from isolated pancreatic islets in vitro is still controversial. Some investigators (3, 4) reported that somatostatin was ineffective in inhibiting insulin release in vitro, while others (5) found it effective. The present study was carried out to elucidate whether somatostatin has direct inhibitory effect on isolated pancreatic islets in vitro. The effect of excessive calcium on somatostatin inhibition of insulin release was also studied in this experiments, since recent investigation (6) with isolated per-

fused rat pancreas have suggested that somatostatin may antagonize the calcium handling in beta cells.

MATERIALS AND METHODS

Male Wistar rats weighing 250-350g, maintained in a temperature-controlled room and fed ad libitum, were used. Two hours prior to the operation, the animals were injected intraperitoneally with 0.2ml of 4% pilocarpine hidrochloride, according to the protocol of Kuo (7). The pancreatic islets of Langerhans were isolated by a slight modification of the technique of Lacy (8). Whole procedures were performed under the ice-cold temperature,except for a period of collagenase digestion and incubation. About 3,800 units of collagenase (Type IV. Worthington Biochemical Co.) were added to 4ml of Krebs-Ringer-Bicarbonate Buffer (KRBB),PH 7.4 , containing chopped pancreas, and the mixture was stirred at 37°C for about 20 min. Careful, sequential observations of digestion mixture by dissection microscope served to determine the timing for discontinuing the collagenase digestion.

Five islets were placed in each vial and preincubated for 30 min with 0.5ml of KRBB containing 3.3mM glucose and 5mg/ml bovine serum albumin (Fraction V. Armour Pharmaceutical Co.), under the atmosphere condition of 95% O_2 and 5% CO_2 at 37°c. The islets were then transferred to fresh KRBB containing various substances and incubated for another 30 min. An aliquot of the medium was removed and immunoreactive insulin were assayed by the polyethylene glycol radioimmunoassay (9), using rat insulin as the standard.

The ionic constituent of the standard KRBB was Na^+ 144.0, K^+ 5.9, Cl^- 128.0, Ca^{++} 2.5, Mg^{++} 1.2, HCO_3^- 25.0, SO_4^{--} 1.2, H_2PO_4 1.2, in mM. In later series of experiments, high calcium medium was employed, in which calcium in the form of $CaCl_2$ was added to the standard KRBB so as to produce a total calcium concentration of 6 mM.

Somatostatin in cyclic form was a generous gift from Prof. H. Yajima (Kyoto University School of Pharmaceutics, Kyoto, Japan). Rat insulin and highly purified pork glucagon were provided by Novo Institute. Dibutyryl cyclic AMP (N^6,0-2-dibutyryl adenosine 3'-5'cyclic monophosphate sodium salt monohydrate) was purchased from Nakarai Chemicals Ltd, Japan.

RESULTS AND DISCUSSION

As shown in Fig. 1, somatostatin caused dose-related inhibition of glucose-induced insulin release from isolated pancreatic islets at the concentrations ranging from 10ng/ml to 10µg/ml. The concentration of somatostatin necessary to suppress insulin release by 50% was approximately 100ng/ml,at the glucose concentration of 16.7 mM.

Addition of 100ng/ml somatostatin also significantly

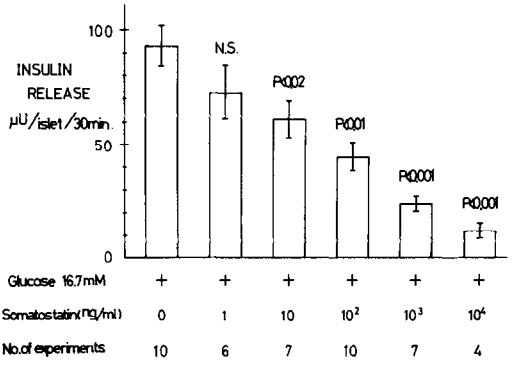

Fig.1: The relationship between the concentration of somatostatin and insulin release from isolated pancreatic islets in the presence of 16.7mM glucose. Mean ± SEM are shown. Statistical differences evaluated by the Student"t" test is shown above each column.

inhibited insulin release elicited by 10μg/ml glucagon or 2mM dibutyryl cyclic AMP, when the calcium concentration was 2.5 mM (Table 1).

The effect of somatostatin was then studied in a medium with high calcium concentration. As shown in Fig. 2, the inhibitory effect of somatostatin on glucose-induced insulin release was completely abolished by raising the calcium concentration from 2.5mM to 6mM. Insulin release at this high concentration of calcium with the addition of somatostatin was comparable to that without the addition of somatostatin (left panel). Excessive calcium also blunted the inhibitory effect of somatostatin on dibutyryl cyclic AMP-induced insulin release. In this case,however, insulin concentration in the vials containing somatostatin was still significantly lower than that in the somatostatin-free vials under the same high calcium concentration. In other words, dibutyryl cyclic AMP-induced insulin release was incompletely restored by excessive calcium (right panel).

Table 1: Effect of somatostatin on insulin release elicited by glucagon or dibutyryl cyclic AMP.

Line	Insulin secretagogues	Somatostatin 100ng/ml.	Insulin release μU/islet/30min. Mean ± SEM.
1.	Glucose8.3mM	-	27.7 ± 3.7 (9)
2.	Glucose8.3mM+Glucagon10μg/ml.	-	76.4 ± 8.1 (7)
3.	Glucose8.3mM+Glucagon10μg/ml.	+	44.7 ± 6.3 (7)
4.	Glucose8.3mM+db-cAMP.2mM.	-	85.7 ±27.8 (5)
5.	Glucose8.3mM+db-cAMP.2mM.	+	20.4 ± 3.3 (9)

Statistical differences by Student "t" test.

Line 1. vs. line 2. $p<0.01$
Line 2. vs. line 3. $p<0.02$
Line 1. vs. line 4. $p<0.02$
Line 4. vs. line 5. $p<0.01$

The numbers of experiments are shown in parentheses.

These results clearly demonstrate the direct inhibitory action of somatostatin on glucose-induced insulin release from isolated pancreatic islets, lending support to the recent publication by Oliver (5). The concentration of somatostatin required for 50% inhibition of insulin release was comparable to that observed in experiments with the perfused rat pancreas (10). This suggest that inhibitory action of somatostatin in vitro is not artifactual caused by the damage of pancreatic islets during the isolation procedure. The reason for controversial results about the inhibitory effect of somatostatin in vitro is still not clear. It is highly likely, however, that collagenase used for the isolation of islets might damage cell membrane under certain condition, thus leading to the loss of responsiveness to somatostatin.

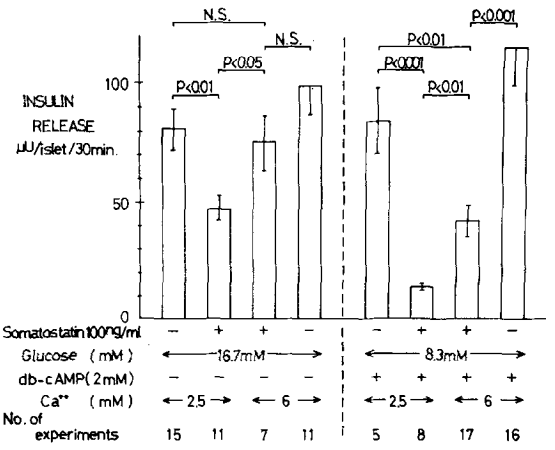

Fig.2: Effect of raising calcium concentration on the somatostatin inhibition of insulin release induced by 16.7mM glucose(left pannel) and 2mM dibutyryl cyclic AMP.(right pannel). Mean ± SEM are shown. Statistical analysis was done by the Student"t" test.

In present experiments, somatostatin also inhibited insulin release elicited by glucagon, which is believed to activate the adenyl cyclase system and, in turn, to stimulate insulin release. This suggest that the inhibitory action of somatostatin on insulin release is not mediated by glucagon. It is likewise observed that insulin release by addition of dibutyryl cyclic AMP was significantly inhibited by somatostatin. These findings indicate that somatostatin might exert its inhibitory effect mainly at distal portions to cyclic AMP production on the secretory process of insulin as suggested by Gerich et al. (11).

Excessive amount of calcium completely abolished the somatostatin inhibition of glucose-induced insulin release, indicating the involvement of calcium mobilization process in the inhibitory effect of somatostatin in pancreatic beta cells. On the other hand, the raised calcium level restored

the somatostatin inhibition of dibutyryl cyclic AMP-induced insulin release only partially. Since both 16.7mM glucose and 2mM dibutyryl cyclic AMP had almost equal insulinogenic activities in somatostatin free system, this disparity might depend upon differences in mode of calcium mobilization or activation in pancreatic beta cells. Malaisse et al.(12) proposed that insulin secretion is always accompanied with two different modesof calcium mobilization in pancreatic beta cells; one, influx from outside to inside through the cell membrane, another, intracellular translocation of calcium from bound to free form. Cyclic AMP is postulated to cause calcium mobilization via intracellular translocation, while glucose cause it mainly via influx through the cell membrane (12, 13). It is possible, therefore, that somatostatin predominantly inhibits the action of cyclic AMP involving intracellular translocation of calcium. However, the possibility that somatostatin acts on more distal points in secretory process could not be ruled out. Further study is required to clarify the detailed mechanisms by which somatostatin inhibits insulin release.

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